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Final Progress Report

for

NASA Contract NAS 9-15566

(1978-1984)

by

W. C. Hymer  
Department of Biochemistry, Microbiology,  
Molecular and Cell Biology  
The Pennsylvania State University  
University Park, PA 16802

(NASA-CR-171846) PURIFICATION AND  
CULTIVATION OF HUMAN PITUITARY GROWTH  
HORMONE SECRETING CELLS Final Progress  
Report, 1978 - 1984 (Pennsylvania State  
Univ.) 26 p HC A03/MF A01

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W. C. Hymer

## Introduction

This report summarizes work done under NASA Contract NAS9-15566 (5/1/78-8/1/84). The PI conferred with Dr. Dennis Morrison in Tucson, Arizona on Oct. 19, 1984 concerning a redraft of this final report. At that time it was deemed appropriate to include only material not covered since submission of my last progress report (i.e. June, 1983).

## Objectives

June 1983 - August 1984.

- 1) To determine if growth hormone (GH) cells contained within the rat pituitary gland can be separated from the other hormone producing cell types by continuous flow electrophoresis (CFE).
- 2) To determine what role, if any, gravity plays in the electrophoretic separation of GH cells.
- 3) To compare in vitro GH release from rat pituitary cells previously exposed to microgravity conditions vs release from cells not exposed to microgravity.
- 4) To determine if the frequency of different hormone producing pituitary cell types contained in cell suspensions can be quantitated by flow cytometry.
- 5) To determine if GH contained within the human post mortem pituitary gland can be purified by CFE.

## Results and Discussion

### Objective #1.

Preliminary evidence for electrophoretic separation of GH cells was accumulated over the past 3 years and was recently published in J. Biophys. Biochem. Methods (Plank et al., 1983; see enclosed reprint). Positive results were achieved using a) density gradient electrophoresis and b) CFE on the McDonnell Douglas (MDAC) device. Results obtained using the latter device are particularly relevant since this is the experimental unit flown on STS-8. A summary of this initial study revealed:

- a) that GH cells were consistently recovered in the high mobility fractions (Fig. 1);
- b) that GH cells, after CFE, were able to produce and release hormone in culture up to 14 days (Fig. 2);
- c) that GH cells did not apparently have unique electrophoretic mobilities as determined by microscopic electrophoresis (Fig. 3) leading to the suggestion that
- d) separations achieved on the MDAC device might be attributed to the high density of the GH cell type ( $1.07-1.08 \text{ g/cm}^3$ ), thus resulting in "fall back" in the device during electrophoresis (Fig. 4).

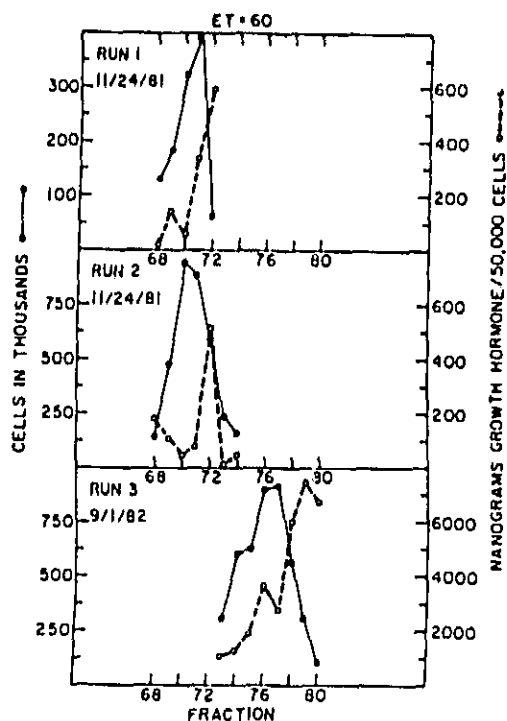


Fig. 1a. Electrophoretic profiles of cells and their GH content in three experiments using the McDonnell-Douglas CFES. In RUN 1 and RUN 2 cells were stored overnight in 0.1% BSA in Medium 199 prior to separation; in RUN 3, freshly prepared cells were used. Inlet was at fraction 59 in all cases. Cell migration was from left to right.

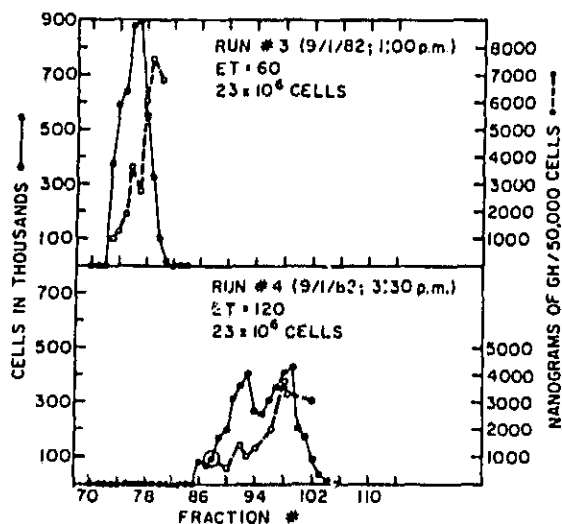


Fig. 1b. Electrophoretic profiles of freshly prepared rat pituitary cells and their GH content in two experiments using the McDonnell-Douglas CFES. The effects of changing ET are seen by comparing RUN 3 with RUN 4.

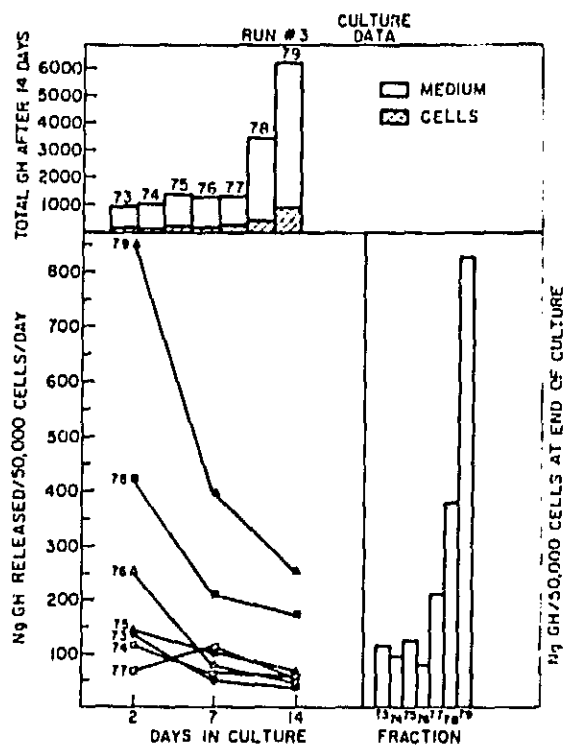


Fig. 2. Production of GH by cells obtained from single electrophoretic fractions after separation using the McDonnell-Douglas CFES. Upper panel shows the total amount of GH that was found in cells and that accumulated in supernatants of the corresponding cultures of each fraction for RUN 3, Figs. 10 and 11. Left panel is the rate of GH production per 50000 cells averaged over 0-2, 2-7, and 7-14 days in culture after electrophoretic separation. Right panel is the total GH production in 14 days per 50000 cells in each fraction.

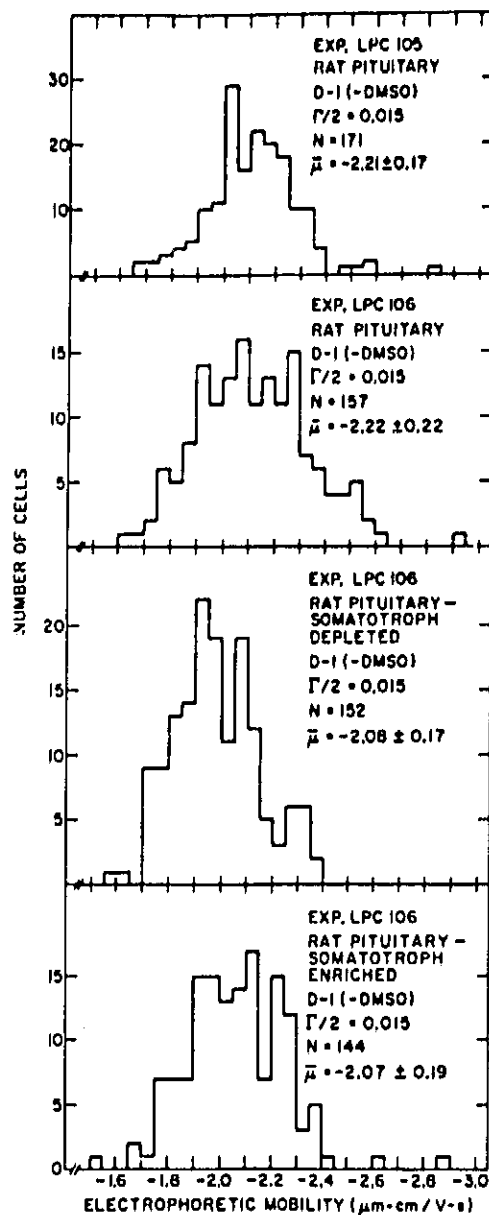


Fig. 3: EPM distributions of suspended rat pituitary cells as determined by microscopic electrophoresis in D-2 buffer ('D-1(-DMSO)') at low (0.015 g-ions/litre) ionic strength. Upper two panels are distributions obtained by examining two different cell pools on different days; reproducibility is shown. Lower two panels are distributions obtained by examining a somatotroph-depleted and a somatotroph-enriched cell population.  $N$  = total number of cells observed.

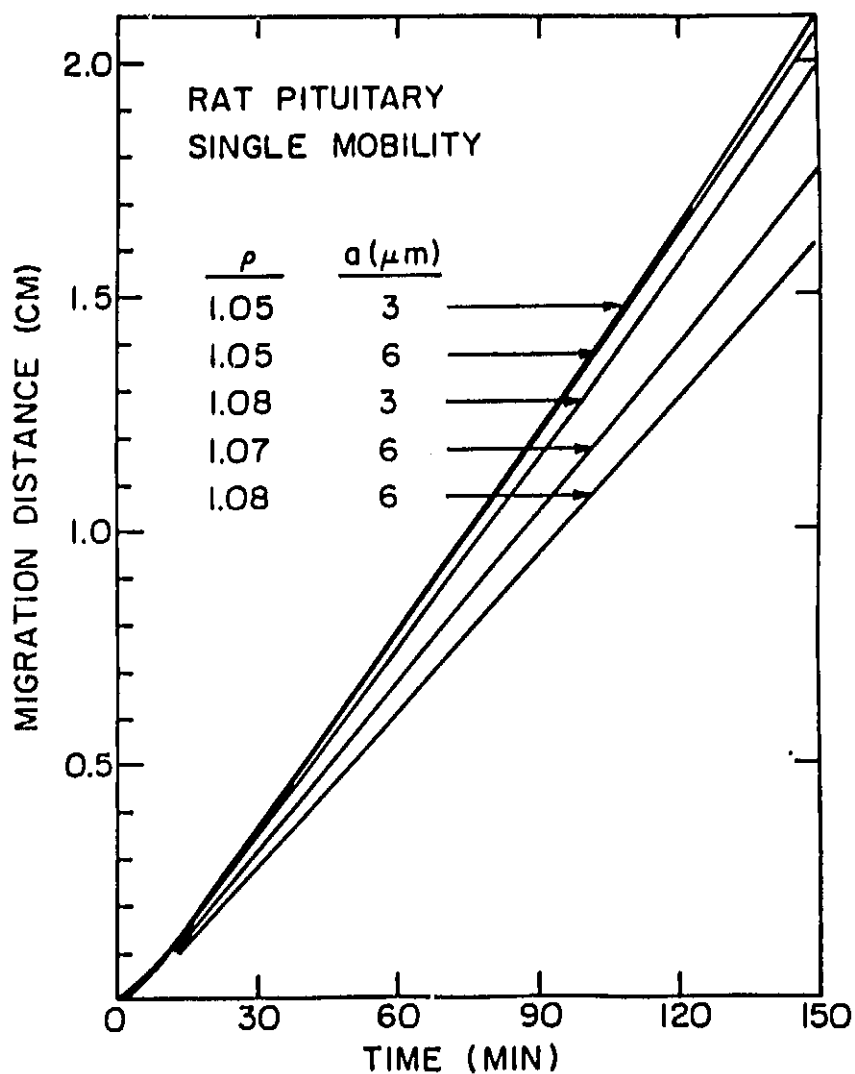


Fig. 4. Migration plots of distance migrated as a function of time in the density gradient column calculated for cells of varying radius and density but all with the same electrophoretic mobility. Values were chosen to be characteristic of known rat pituitary cell subpopulations, and a linear dependence of EPM on Ficoll concentration was assumed.

The results from this initial study were important for the following reasons:

first; prior to publication of these results were no reports in the literature indicating that electrophoretic separation of different rat pituitary cell types had ever been attempted or achieved;

second; our experimental evidence (Fig. 1, 2) was consistent with the hypothesis that GH cells had a unique surface charge density and

third; the possibility of density-driven separations of GH cells during CFE appeared to provide an excellent rationale for a CFE experiment in microgravity.

#### Objective #2.

As explained above, a CFE experiment in microgravity was done to find out if cell density played a role in our ground based CFE experiments. To our way of thinking there were 2 possible outcomes of such an experiment:

Outcome #1. Result: similar to ground based data.  
Interpretation: cell density does not play a role in separations on CFE and GH cells do indeed have unique surface charge densities.

Outcome #2. Result: no separation of GH cells, i.e. different result from ground based experiments.  
Interpretation: GH cell density does play a role in CFE separations; therefore GH cells do not have unique surface charge densities.

The reader will appreciate that the logistics of conducting a microgravity CFE experiment are unique and somewhat difficult. To control for pre and post-processing holding times we carried out additional ground based studies before and after the flight of STS-8. Holding conditions, as well as parameters tested in these experiments, are given in Table 1. The actual experimental protocol for the microgravity experiment is outlined in Table 2 and "event" times are outlined in Table 3.



Table 1  
RAT PITUITARY CELL SEPARATION ON CFES: PROTOCOLS

Exp	Gravity Condition	Hours/Days				Parameter <sup>+++</sup>			
		ET	BE <sup>+</sup>	AE <sup>++</sup>	ICH	Intracell Hormone	Cult	HPLC	SDS-PAGE
GS-1	1	150	40 hr	6 days	0	+	+	0	0
GS-2	1	210	0.5 hr	0.5 hr	+	+	0	0	0
GS-3	1	150	72 hr	5 days	+	+	+	+	+
STS-8	Micro	300	72 hr	5 days	+	+	0	+	+
GS-4	1	150	0.5 hr	0.5 hr	0	+	+	+	+

<sup>+</sup>Approximate time between rat kill and electrophoresis.

<sup>++</sup>Approximate time between electrophoresis and cell processing.

<sup>+++</sup>ICH = immunocytochemistry; Intracellular hormone = RIA/EIA on 0.01 N H<sub>2</sub>CO<sub>3</sub> extracts; culture = MEM + 5% calf serum + antibiotics; HPLC = reverse phase on C-18 column.

Table 2  
STS-8 PROTOCOL

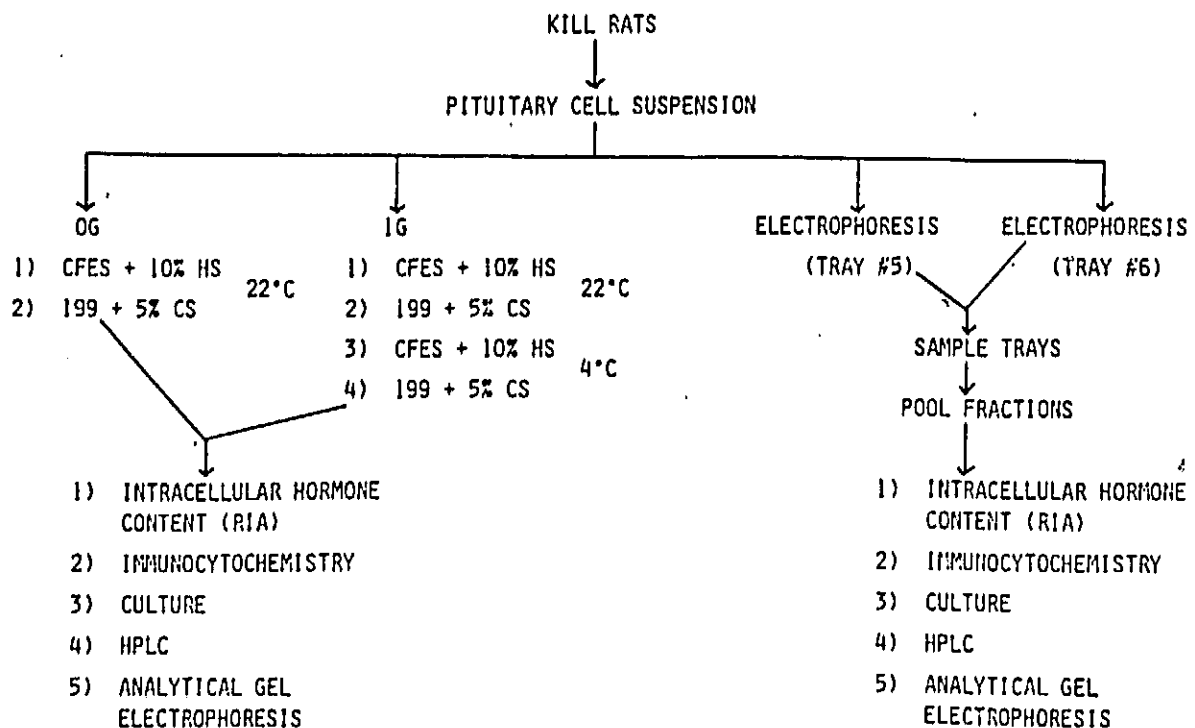


Table 3

## STS-3 PITUITARY CELL ELECTROPHORESIS EXPERIMENT

TASK	DATE	TIME FROM KILL	
		HOURS	DAYS
1) KILL RATS	8/29	-	-
2) PREPARE CELL SUSPENSION	8/29	4	-
3) CELLS TO CAPE	8/29	8	-
4) CELLS INTO CFES SYRINGES	8/29	10	-
5) ELECTROPHORESIS STS-8	9/1	-	3
6) LANDING	9/5	-	8
7) BEGIN SAMPLING TRAY #5	9/5	-	8
8) END SAMPLING TRAY #5	9/6	-	9
9) PROCESS CONTROLS TRAY #5	9/6	-	9
10) BEGIN SAMPLING TRAY #6	9/7	-	10
11) END SAMPLING TRAY #6	9/7	-	10
12) PROCESS CONTROLS TRAY #6	9/8	-	11

Results of 4 CFE expts., viz. GS-2, STS-8, GS-3, and GS-4 (our experimental designation) are presented graphically in Fig. 5 as expts. 1, 2, 3 and 4 respectively. Clearly, GH cell separation occurred in microgravity (exp. 2), thereby confirming that GH cells do indeed possess unique surface charge. (Note: the reason these experimental results are "crowded" into one figure is simply that they are being submitted for publication in Science in this format where space is at a premium).

Fig. 5.

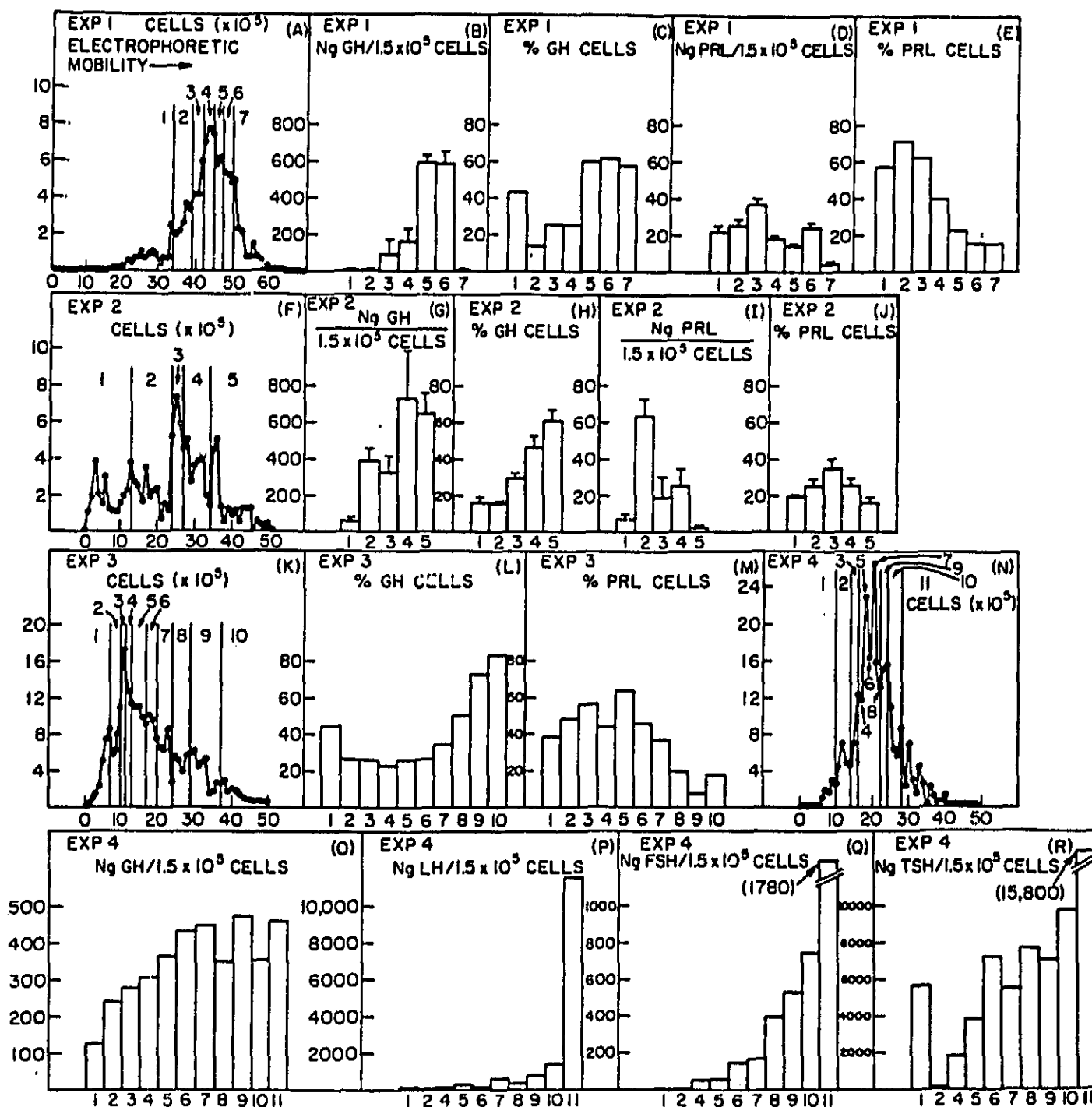


Fig. 5 Results of 4 rat pituitary cell CFE experiments done under unit gravity (experiments 1,3,4) or microgravity (experiment 2) conditions. Numbers of anterior pituitary donors (250 g Sprague Dawley derived rats, Hilltop Labs., Scottsdale, PA) used in experiments 1-4 were 50, 134, 50, and 73 respectively. Tissues were dissociated into single cells with a trypsin-albumin solution (2) and washed 2x (500  $\times$ g, 10 min, room temperature) in CFE buffer. At this stage cell viability averaged  $90 \pm 5\%$ . In experiments 1 and 4 cells were injected into the CFE device immediately after the CFE buffer wash; in experiments 2 and 3 the cells were held at  $4^{\circ}\text{C}$  for 3 days in 5 ml syringes prior to injection. ET values (see pg. ) were 210, 150, 150 and 150 volt-min- $\text{cm}^{-1}$  for expts. 1-4 respectively. Fifty-80 ten ml fractions were collected into tubes containing 3 ml Alpha modified Eagle's medium and calf serum (Gibco) such that the final concentration was 1x Alpha modified Eagle's medium + 5% calf serum + penicillin-streptomycin 100 U/ml + gentamycin sulfate 10  $\mu\text{g}/\text{ml}$  (experiments 2 and 3 only). Cells in experiments 1 and 4 were washed in MEM containing 0.1% BSA immediately after collection; those in experiments 2 and 3 were held at  $4^{\circ}\text{C}$  for 5 days prior to washing and counting. Individual fractions were pooled according to the scheme shown in the first panel of each of the 4 experiments. The average number of cells in each pooled fraction ( $\pm$  SEM) was  $1.51 \pm 0.09 \times 10^6$  (Exp 1);  $2.22 \pm 0.12 \times 10^6$  (Exp 2);  $2.52 \pm 0.14 \times 10^6$  (Exp 3) and  $1.72 \pm 0.10 \times 10^6$  (Exp 4). Cells contained in each of these pooled fractions and in the CFE-washed, non-electrophoresed fraction were analyzed a) by immunocytochemistry to determine the percentage of different cell types (experiments 1, 2, 3); b) by specific, homologous radioimmunoassays to determine intracellular hormone levels (experiments 1, 2 and 4) and hormone levels after cell culture (experiments 3, 4) and c) by HPLC for characterization of intracellular GH (experiments 2 and 4). Procedures for immunocytochemistry and antiserum controls are described in (8). Antisera final dilutions: anti-GH, 1:50,000; anti-PRL, 1:50,000; anti-LH, 1:10,000. Differential cell counts were obtained on 500-1000 cells on coded slides by 2 or 3 investigators. The average % of GH, PRL and LH cells contained in the CFE washed, nonelectrophoresed cells was  $35 \pm 6\%$ ,  $20 \pm 5\%$ , and  $10 \pm 3\%$  respectively for experiments 1, 2 and 3. Intracellular hormone levels were measured on 0.1 M  $\text{NaHCO}_3$  extracts using RIA kits kindly provided by the National Institute of Arthritis, Digestive Diseases, and Kidney NIADDK (FSH, LH, TSH) or previously validated RIA's [GH, PRL-(9)]. Results are expressed in terms of standards designated as follows; GH VII-38C; PRL 10-10B; LH RP-1; FSH RP-1; and TSH RP-1. Electrophoretic cell profiles for each of the experiments are numbered according to where cells first appeared among harvested fractions and are not defined according to actual electrophoresis channels. Cell viabilities after electrophoresis were 80%, 53%, 56%, 74% in experiments 1-4 respectively. Numbers of cells electrophoresed were  $4.9 \times 10^7$ ,  $5.38 \times 10^7$ ,  $3.47 \times 10^7$  and  $5.15 \times 10^7$  in experiments 1-4, respectively. Cell recoveries were 21%, 20%, 73%, and 48% in experiments 1-4. Low recoveries are attributed to cell clumping in the holding syringe used for injection into the CFE device. The total number of any given hormone-containing cell type contained in a pooled fraction is obtained by multiplying the % of immunopositive cells in that fraction (panels C, E, H, J, L, M) by the total number of cells in that pooled fraction (see above). Thus, 63% of the total recovered GH cells in Exp 1 were found in pooled fractions 5, 6 and 7; 67% of the total recovered PRL cells in Exp 1 were found in pooled fractions 1, 2 and 3. On the basis of both immunocytochemistry and immunoassay data, there was no indication of preferential cell loss during electrophoresis.

Counts on immunochemically stained cells contained within the various fractions after CFE were critical to document cell enrichment. Examples of the cell appearance in these different experiments are offered in Fig. 6. Since cells are necessarily subjected to harsh conditions (e.g. low ionic strength buffers, long storage at 4°C, etc.), it is important to document that we still have intact cells after these experimental manipulations. The images in Fig. 6 clearly show that this is the case.

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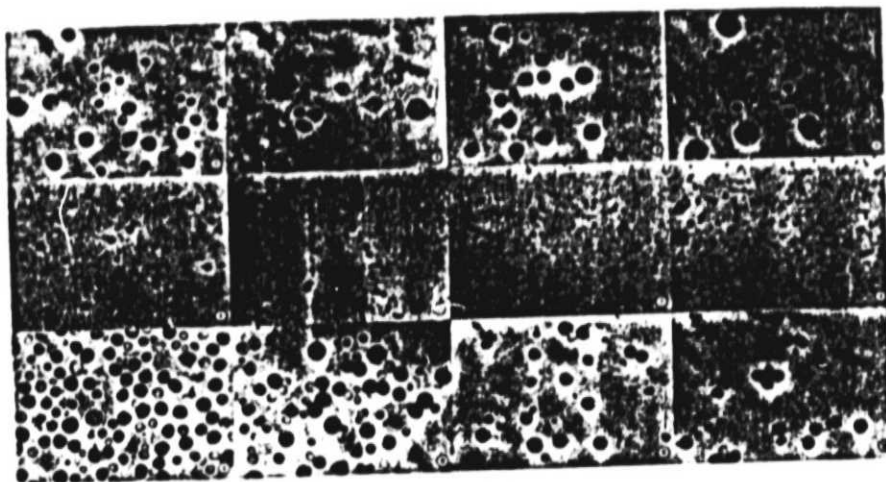


Fig. 6 Morphology of pituitary cells before and after separation on CFE. Cells were deposited on polylysine-coated cover slips and stained for intracellular hormone (8). Preabsorption of antisera with excess hormone abolished staining. Cells were also unstained when nonimmune sera were used at comparable dilutions. Panels 5-8, x 100; all others x 620. Panels 1-8 show selected cell fractions obtained from CFE experiment 1; panels 9-12 from CFE experiment #2 (see Fig. 2). In all cases darkly stained cells are hormone specific while nonstained cells are grey. Panel 1, GH cells in initial, non-electrophoresed preparation; panel 2, non-GH cells in electrophoresis fraction 4; panel 3, GH cells in fraction 6; panels 4 and 5, LH cells in initial non-electrophoresed preparation at high (620x) and low magnification (100x); panel 6, LH cells in fr. 7; panel 7, PRL cells in initial non-electrophoresed preparation; panel 8, PRL cells in fr. 2; panel 9, GH cells in non-electrophoresed preparation before CFE buffer wash; panel 10, GH cells in non-electrophoresed preparation after CFE buffer wash; panels 11 and 12, GH cells in frs. 4 and 5, respectively.

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We were especially interested to find out if cells recovered from the CFE device were able to produce and release hormone in culture over prolonged periods. If they did, cell viability during and after CFE would be demonstrated. Results of such attempts (Fig. 7) show

- that GH cells continue to release hormone for at least 12 days in vitro and
- that GH cells are separated from PRL cells (which also continue to release hormone).

Fig. 7

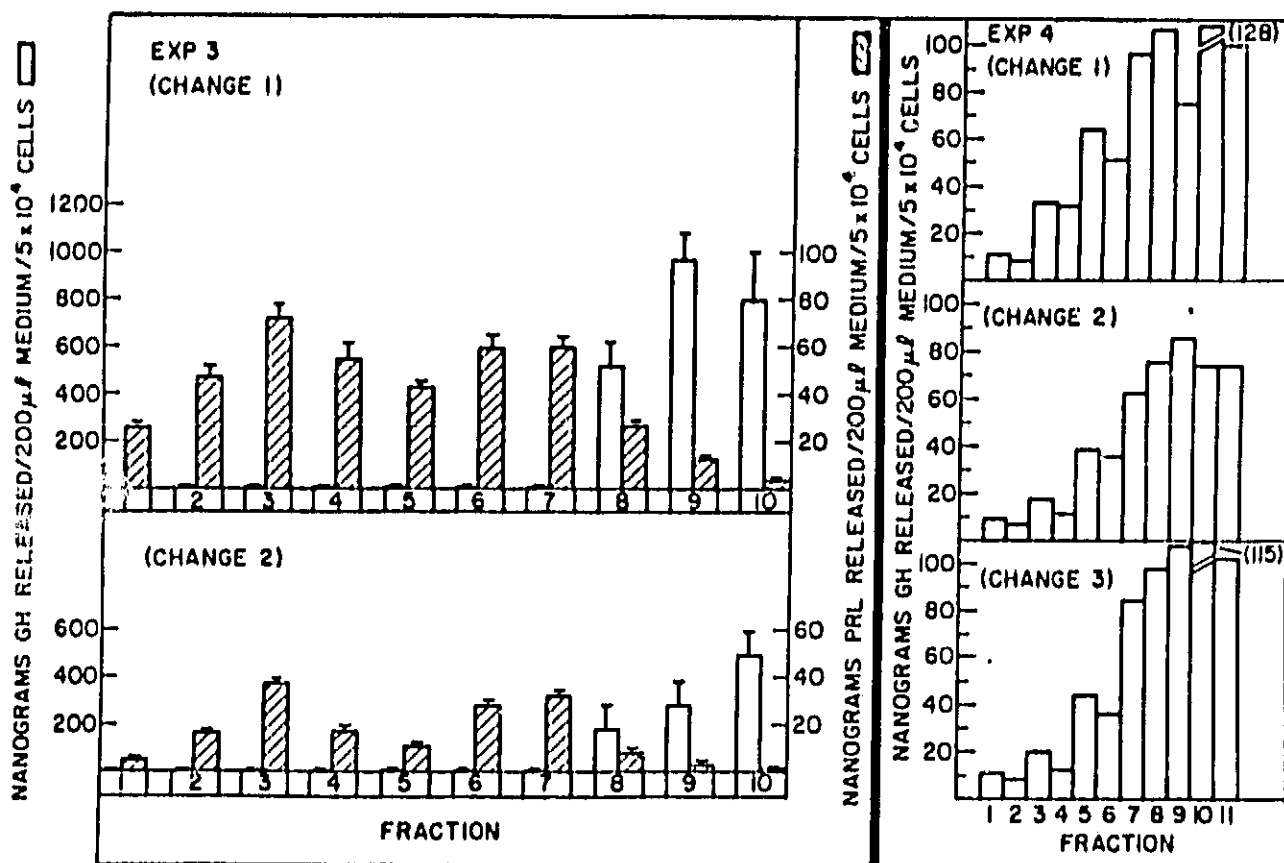


Fig. 7 In vitro release of immunoreactive PRL and GH from pituitary cells after separation by CFE. In experiment 3,  $5 \times 10^4$  cells from each of the 10 CFE fractions (Fig. 2, panel K) were cultured in 200  $\mu$ l alpha MEM containing 5% calf serum and antibiotics in 96 well plates (5 wells/fraction) at 37°C under 95% air + 5% CO<sub>2</sub>. Total culture time was 8 days with 1 medium change at day 4. In experiment 4,  $75 \times 10^5$  cells from each of the 11 CFE fractions (Fig. 2, panel N) were cultured in single T-25 flasks containing 5 ml of the same culture medium. Total time in culture was 12 days with medium changes at day 4 and 8. In addition to PRL and GH RIA's (see Fig. 2 legend), medium in experiment 4 was tested for the presence of bioactive GH using the tibial line assay of Greenspan et al. (10). (See Table 1).

The reader will recognize that Fig. 7 does not contain culture information from the microgravity experiment. Unfortunately, cells from microgravity were heavily contaminated from the MDAC-CFE device. Two major problems associated with the STS-8 experiment were a) the bacterial contamination and b) loss of cells attributed to settling in the sample syringe, which forced large "pooling" of cells recovered from the CFE unit.

We have been analyzing intracellular forms of the GH molecule contained within CFE fractions by HPLC. This powerful analytic technique permits analysis of hormone size and shape depending upon the particular HPLC column used. Shown in Fig. 8 are the results of the size of intracellular GH molecules contained in CFE fractions in exps. 2 and 4. Clearly, most of the hormone appears to be monomeric (22K) GH. On the other hand, our preliminary data obtained using HPLC-reverse phase columns support the hypothesis that different GH cells contained within different CFE fractions contain hormone with different shapes (Fig. 9). These results are very exciting and completely novel. However, more work will be required for their verification.

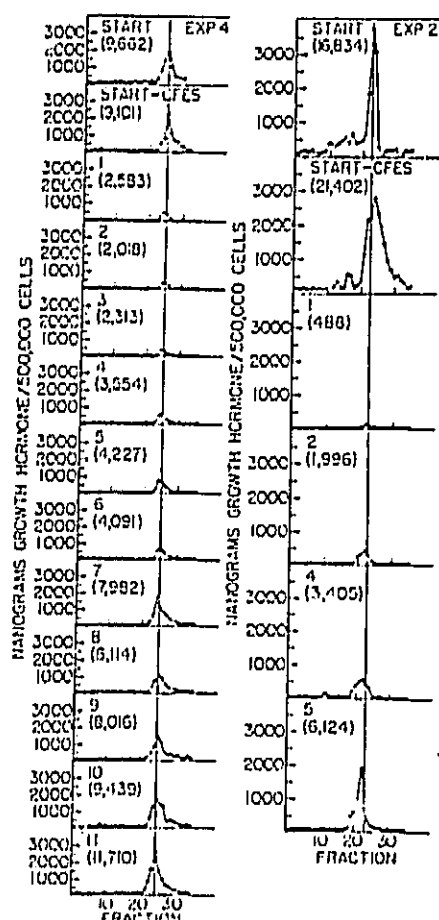
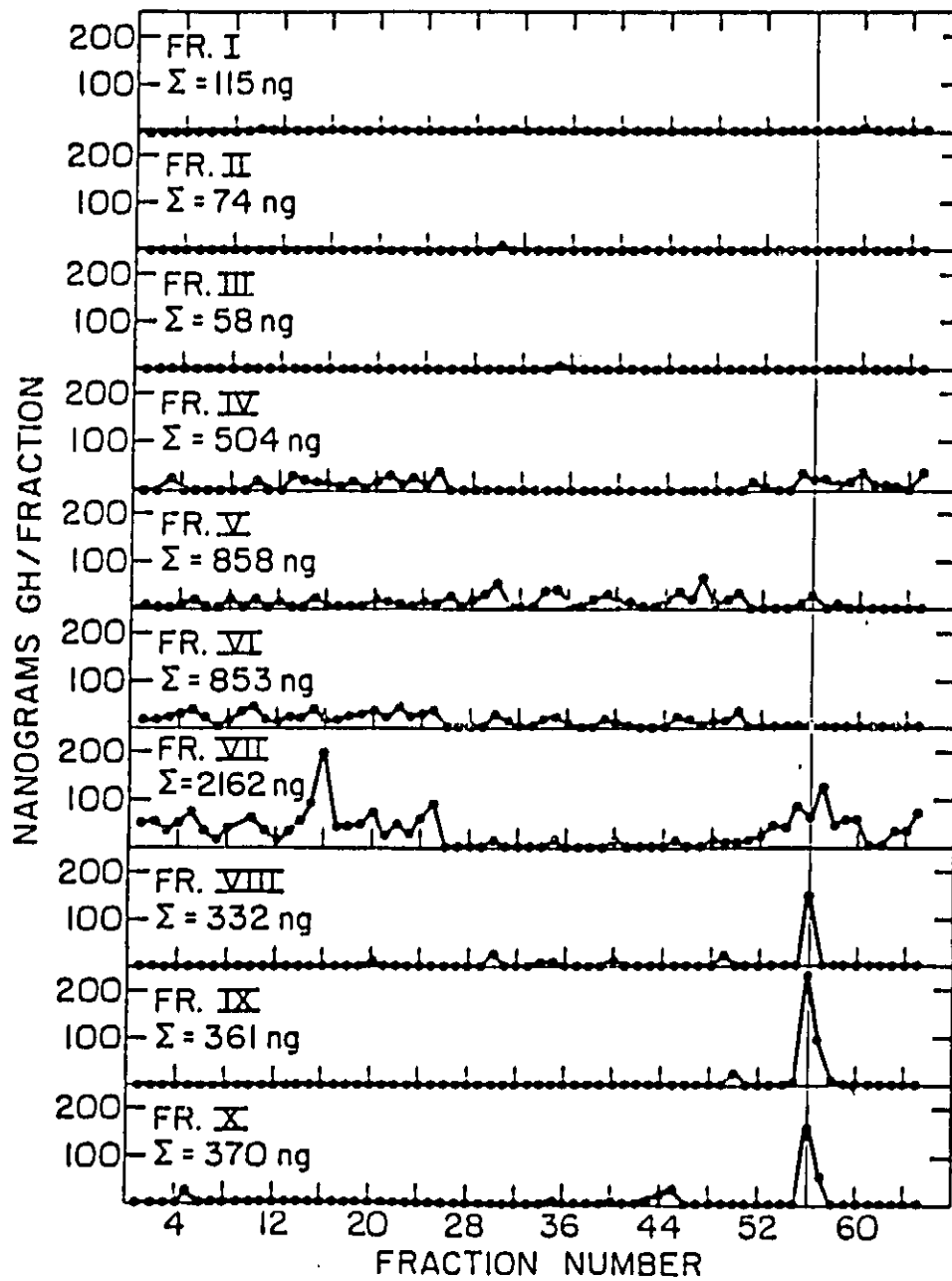


Fig. 8

Fig. 8. Profiles of intracellular GH extracted from CFE fractions (experiments 2 and 4, see Fig. 1) after HPLC on 2x Waters Progein Pak 300 SW sizing columns. GH contained in each of the 36 HPLC fractions was measured by specific GH RIA. Molecules in the size range of monomeric GH ( $M_r=22K$ ) elute in fractions 22-24. The total amount of GH measured in each HPLC run is given in parentheses. Start and Start-CFES refers to dissociated, but not electrophoresed cells before and after CFES (continuous flow electrophoresis system buffer wash). Low hormone recoveries in experiment 2 are attributed to release of GH from cells held at 4°C for 5 days in after CFE.

Fig. 9





Finally, in collaboration with Dr. Richard Grindeland of NASA-Ames, we have begun to investigate the issue of the biological/immunological activity ratios of GH secreted from cells after CFE. The recent, unpublished results of B/I activities of GH in media from exp. 4 (Table 4) clearly document important fundamental differences in these parameters and invite further study.

Table 4  
Bioactive Rat GH released into culture media  
from CFE separated cells in experiment 4.

Combined Electrophoresis Fraction <sup>+</sup>	<u>µg Released Bioactive GH/Combined Fraction/750,000 Cells</u>			
	Change 1 <sup>++</sup>	Change 2	Change 3	Sum
1+2	0	3.7	3.1	6.8
3+4	0	3.1	4.0	7.1
5+6	0	8.6	9.9	18.5
7+8+9	23.2	29.8	18.1	71.1
10+11	27.0	40.7	20.3	88.0
Start-Buffer <sup>+++</sup>	24.9	27.5	12.9	65.3
Start-Media	20.7	51.6	11.8	84.1

<sup>+</sup> See Fig. 1 (panels N, O) for electrophoresis cell profile and intracellular GH levels obtained in experiment 4. See Fig. 3 for levels of immunoassayable GH released into the culture medium from the 11 individual fractions in experiment 4.

<sup>++</sup> 750,000 cells in each electrophoresis cell fraction were individually cultured for a total of 12 days with 2 intervening changes at day 4 and 8. After GH RIA of individual fractions (Fig. 3), medium samples were pooled as indicated and assayed by tibial line bioassay (7) using 2 x 2 design (4 hypophysectomized ♀ rats/point). Bovine GH, 1.5 IU/mg, covering the dose range of 5-45 µg, used as standard (n=5 animals/point). The values obtained against bovine GH standards were converted to values for rat GH (3 IU/mg).

<sup>+++</sup> Aliquots of the initial cell suspension which were not electrophoresed but kept at 4°C in either CFE buffer or medium 199 containing 0.1% bovine serum albumin for a time period comparable to the length of the CFE run.

It should be obvious to the reader that a great deal of work has been done to meet Objective #2. In our view the microgravity CFE experiment was ~80% successful. In a future microgravity experiment we would like to be able to recover large numbers of uncontaminated cells to find out what effect the microgravity environment may have on subsequent GH production/release at both the cellular and molecular level.

A comparison of the experimental results obtained on STS-8 vs ground based control is given in Table 5.

Table 5  
Comparison of data obtained in ground based and microgravity based CFE pituitary cell separation experiments.

<u>PARAMETER</u>	<u>UNIT GRAVITY</u>	<u>MICROGRAVITY</u>
1) Cell profile after electrophoresis	1) spread over ~50 tubes	1) spread over ~50 tubes
2) Cell enrichment-immunocytochemistry	2)	2)
a. GH	a. 2-3x enrichment in mobile fractions	a. 2x enrichment in mobile fraction
b. PRL	b. 3x enrichment in low mobility fractions	b. ~2x enrichment in low mobility region
c. LH	c. 2x enrichment in most mobile area	c. 2x enrichment in most mobile area
3) Cell enrichment-intracellular hormone (RIA)	3) parallels immunocytochemistry	3) parallels immunocytochemistry
4) GH cell culture (RIA)	4) GH released from both high and low mobility somatotrophs	4) GH not released (?)
5) GH cell culture (Bioassay)	5) bioactive GH preferentially released from high mobility fractions	5) not done
6) LH, FSH, TSH cell culture (RIA)	6) a. LH and FSH output from more mobile cells b. TSH-no evidence for separation c. PRL-output from less mobile cells	6) not done
7) Character of GH after HPLC on gel sizing columns	7) a. $M_r$ GH=22K for all regions b. more GH from high mobility regions	7) a. $M_r$ GH=22K for all regions b. more GH from high mobility regions
8) Character of GH after HPLC on reverse phase columns	8) Evidence for multiple GH forms in somatotroph subpopulations	8) Evidence for multiple GH forms in somatotroph subpopulations

### Objective #3.

The "left leg" of our microgravity experimental protocol (Table 2) reflects the fact that we were able to store dispersed rat pituitary cells in a Shuttle Middeck storage locker and subsequently culture them on earth. It is emphasized that these cells were not subjected to CFE.

The data in Table 6 provide the evidence which suggests that microgravity-exposed GH cells are unable to release as much hormone as their corresponding earth-based controls. Thus, GH production (i.e. GH in medium intracellular GH) was 20.3 times greater from control cells than from those exposed to microgravity (Table 6). Most interestingly, PRL secretion was affected in exactly the opposite way. Thus, PRL release was 4x greater from the same microgravity exposed cells (Table 6).

Table 6  
GH AND PRL PRODUCTION BY RAT PITUITARY CELLS IN CULTURE AT UNIT GRAVITY  
AFTER PREVIOUS EXPOSURE TO MICROGRAVITY OR UNIT GRAVITY CONDITIONS.

Gravity Condition (xg)	Temp °C	Intracellular GH (ng/1 x 10 <sup>6</sup> cells)	Total GH released from 1 x 10 <sup>6</sup> cells during 6 day culture (ng) <sup>+</sup>	ng GH Produced during 6 days <sup>++</sup>
Micro	AMB	540 ± 70	765	225
1	AMB	3,850 ± 1,410	16,760	12,910
1	4	11,700 ± 3,240	21,050	9,350

Gravity Condition (xg)	Temp °C	Intracellular PRL (ng/1 x 10 <sup>6</sup> cells)	Total PRL released from 1 x 10 <sup>6</sup> cells during 6 day culture (ng) <sup>+</sup>	ng PRL Produced during 6 days <sup>++</sup>
Micro	AMB	64 ± 5	5,025	4,961
1	AMB	1,702 ± 395	3,275	1,573
1	4	2,051 ± 218	3,600	1,549

<sup>+</sup> 1 x 10<sup>6</sup> cells in 5 ml αMEM + 5% CS + Antibiotics. Represents total hormone in medium detected in two media changes.

<sup>++</sup>Ng hormone produced = hormone released - hormone intracellular at seeding.

Is there precedent for microgravity-induced effects at the cellular level? Yes. The recent data of Cogoli (1984; Science) shows that lymphocytes exposed to ConA in space do not respond in the usual fashion. The implication(s) of "gravity sensors" within cells is discussed in a manuscript and talk presented at JPL in Pasadena last December, 1984 (enclosed). A proposal describing experiments to further confirm and investigate this microgravity induced "secretory lesion" was submitted to NASA-Life Science in April, 1984.

#### Objective #4.

The mammalian anterior pituitary is noted for extreme heterogeneity of hormone-containing cell types. The investigator interested in separating these various cell types by physical means is, of course, required to monitor the quality of those separations by morphological methods. Currently, the most sensitive and specific of these is immunocytochemistry. This technique is based on the ability of highly specific, non cross-reacting hormone antibodies to recognize intracellular antigens at high dilutions of antisera (typically 1:50,000). After incubation with a second antibody system constructed to provide a staining signal (e.g. either fluorescence or enzyme-linked conjugates) the frequency of specific cell type in a given fraction must be counted manually under the microscope to obtain quantitative information on the frequency distributions of that particular cell type.

A typical CFE cell separation experiment will yield 25-50 individual fractions for staining and subsequent microscopic counting for each of the 6 pituitary hormones. Our usual procedure calls for random counting of 500-1000 stained cells on coded slides by 2-3 investigators. As each slide requires a minimum of 20' to count, the amount of time and effort invested to get the job done is clearly not trivial.

Approximately 1.5 years ago our Department was given NIH support to buy a flow cytometer. Mike Hatfield, a graduate student in my laboratory, has developed a method to count the different pituitary cell types contained in pituitary cell suspensions by flow cytometry. Basically the method involves staining of ~200,000 fixed cells in suspension using primary antiserum followed by incubation in fluorescein labeled secondary antibody. The laser rapidly "counts" stained vs unstained cells so that 10,000 cells can be scored in a very few minutes. A manuscript describing this methodology, together with data on distributions of GH, PRL and LH containing cells, is currently in press (Flow Cytometry -see Appendix). While we have not yet used the method on cell fractions obtained from the MDAC-CFE device, we are eager to do so in the future.

Finally, we have begun to explore the possibility that specific hormone producing cell types have internal structures which can serve as unique "fingerprints" when the laser interacts with the unstained cell. Our data indicate that GH cells, by virtue of their extensive complement of hormone-containing secretory granules, show a high 90° scatter component. In theory we should be able to sort cells on the basis of this scatter pattern and evaluate them for B/I GH production.

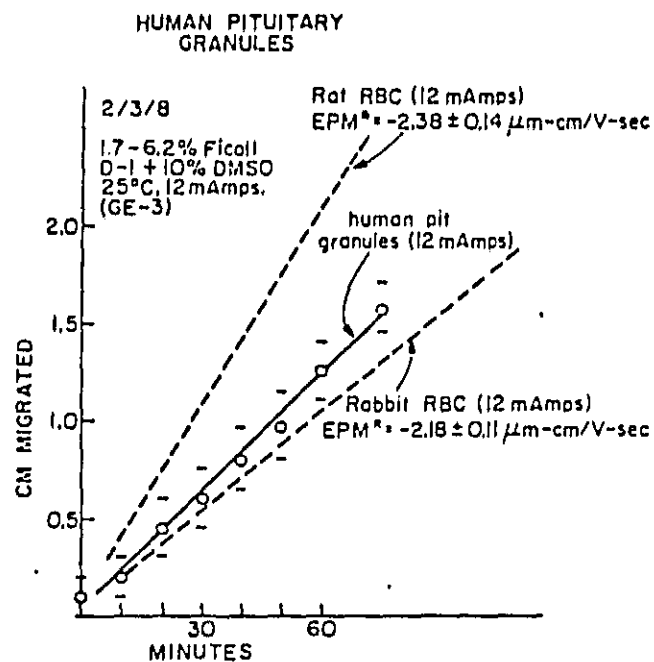
Objective #5.

Growth hormone cells are noted from their high numbers of cytoplasmic, hormone-containing, secretory granules. A major percentage of the total intracellular hormone is, in fact, associated with these particles. We have been interested in isolating GH from these particles using the CFE device to purify the granules prior to hormone extraction. Our reasoning has been that heterogeneity exists within the secretory granule populations, and that some GH granules contain hormone with higher B/I activity ratios than others. Further we hypothesize that subpopulations of GH-containing granules bear different surface charge densities on their limiting membranes and, as a result, have different electrophoretic mobilities.

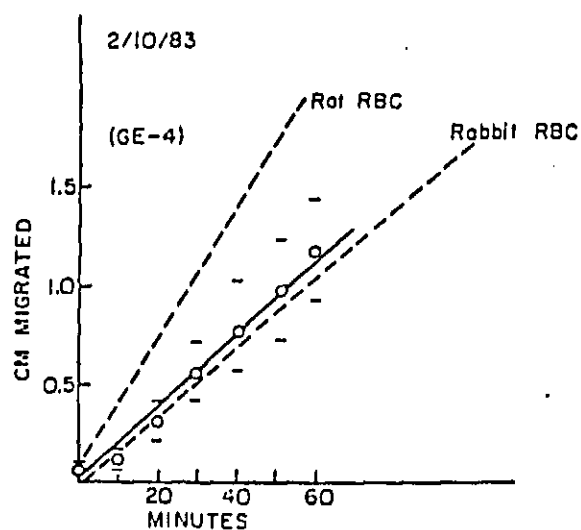
Since it is well known that GH granules in the human pituitary resist autolysis for at least 24 hrs. after death, we have been particularly interested in applying this approach to the human post mortem pituitary gland. If we could purify high potency hormone from this source, it could certainly be used for therapeutic purposes.

That human GH secretory granule bear a surface charge is suggested by the data in Fig. 10. These show that such particles have a mobility, determined by gradient density electrophoresis, somewhere between rat and rabbit RBC's.

Fig. 10



\* determined by microelectrophoresis



The protocol currently in use to prepare a crude human GH secretory granule fraction is given in Fig. 11. Most experiments use the "left leg" of this protocol, i.e. electrophoresis of the particle followed by solubilization for hormone assay. Results from a recent experiment, shown in Fig. 12, indicate that immunoreactive GH continued in the gland of a 11 hr. post-mortem specimen is associated with particles which had migrated to positions 100-109. However, results from a similar experiment utilizing material from a 8 hr. post-mortem gland show that GH is much more heterogenous in this preparation, being distributed over fractions 91-140 (Fig. 13). We interpret this result to indicate that some damage had occurred to the GH in this tissue.

Individual tubes from the experiment depicted in Fig. 12 were pooled according to the following scheme 0-89 (I) 90-103 (II), 104-109 (III), 110-200 (IV). B/I activities of GH in these fractions were I = 0; II = 2.4; III = 2.8; IV = 62.8.

We believe that these results are especially important to the issue of heterogeneity of GH in the human pituitary and to processing of pooled samples of glands. If the distributions in 2 human pituitaries are so different, it systems probable that hGH preparations currently administered to patients represent a mixture of electrophoretically distinct hormone species. Obviously, more work is required to characterize hGH from this source of using this unique approach.

Fig. 11

CFES PROTOCOL FOR HUMAN POST MORTEM GRANULES/EXTRACTS

AT 1 G AND MICROGRAVITY

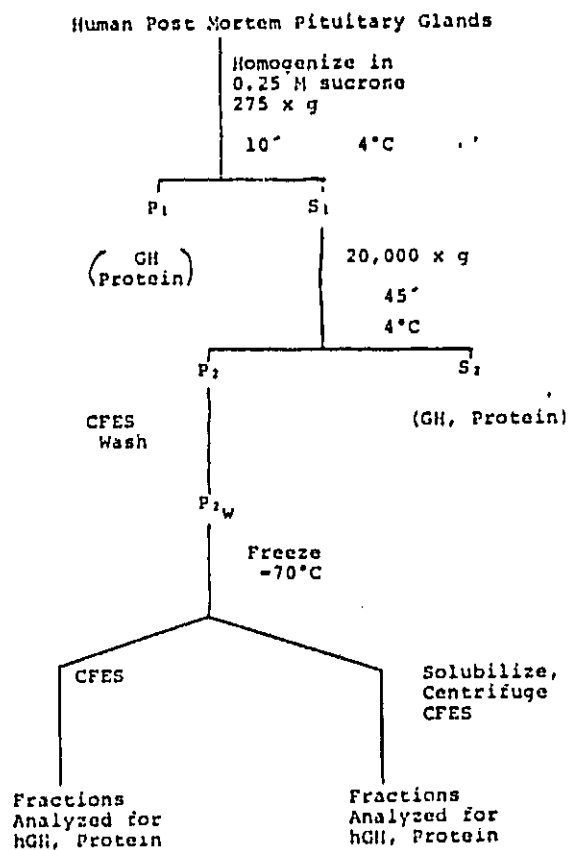




Fig. 12

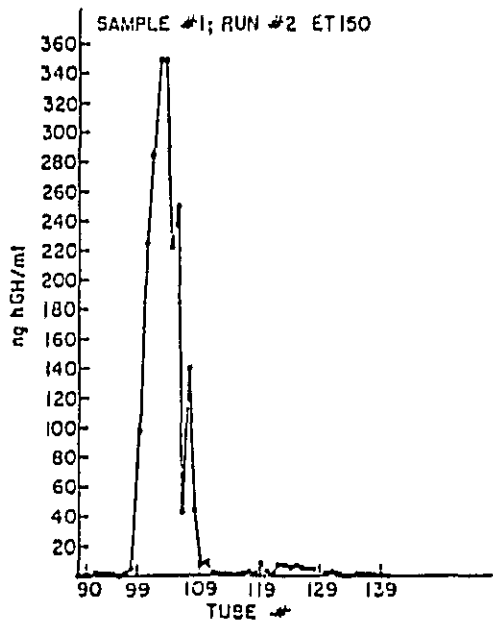
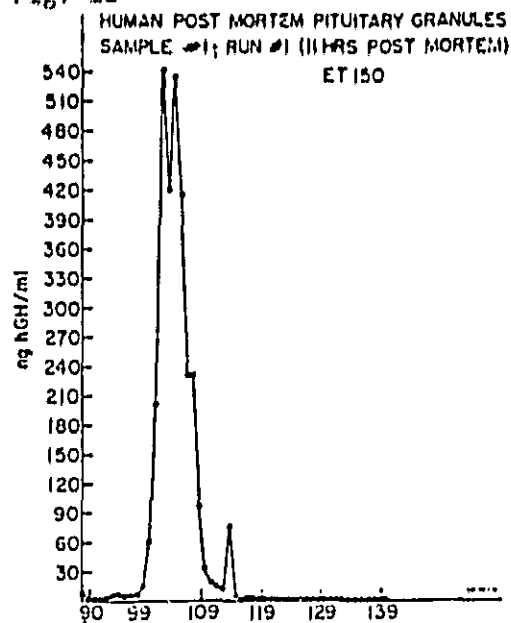
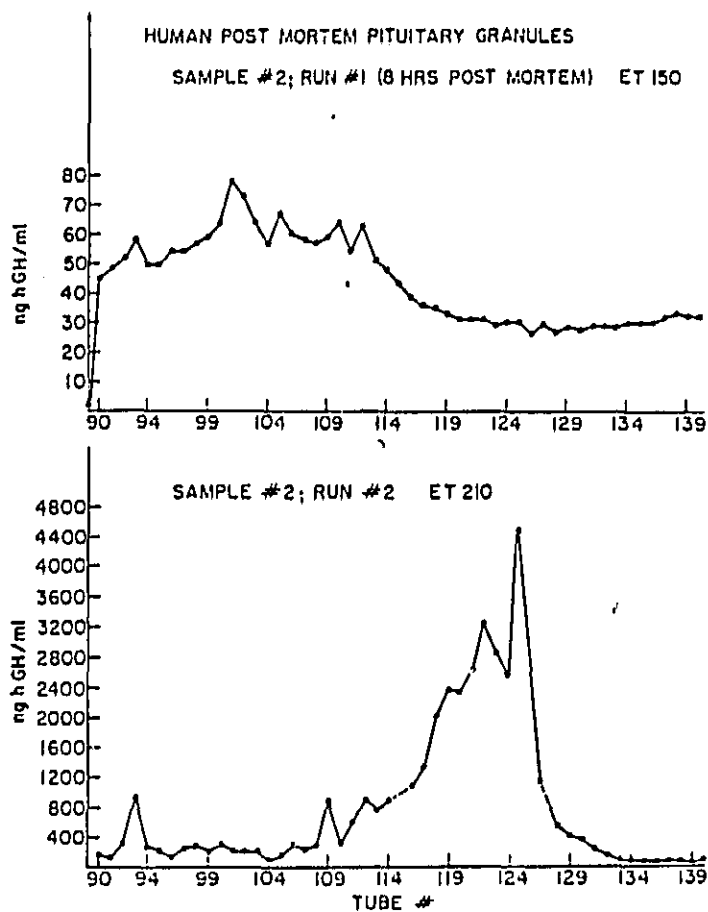


Fig. 13



Publications Credited to NAS9-15566 (1978-1984)

Hymer, W. C., D. Wilbur and A. Dekker. 1980. The human post mortem pituitary contains functional somatotrophs. Proc. 62nd Meetings Endocrine Soc. p. 241 (665).

Hymer, W. C., R. Page, R. Kelsey, E. Augustine, W. Wilfinger and M. Ciolkosz. 1980. Separated somatotrophs: Their use in vitro and in vivo. in Synthesis and Release of Adenohypophysial Hormones, eds. N. Jutisz and K. McKerns, Plenum Press, pp. 125-166.

Hymer, W. C. and D. Wilbur. 1980. Dispersed pituitary cells: Their use in vitro and in vivo. in Functional Correlates of Hormone Receptors in Reproduction, eds. V. Mahesh, T. Muldoon, B. Saxena and B. Sadler, Elsevier North Holland, pp. 13-44.

Hymer, W. C., D. Wilbur, R. Page, E. Hibbard, R. Kelsey, J. Hatfield. 1981. Pituitary Hollow Fiber Units in vivo and in vitro. Neuroendocrinology 32:339-349.

Hymer, W. C., J. Harkness, A. Bartke, D. Wilbur, J. Hatfield, R. Page and E. Hibbard. 1981. Pituitary Hollow Fiber Units in the Dwarf Mouse. Neuroendocrinology 32:350-354.

Kelsey, R., W. C. Hymer and R. Page. 1981. Pituitary Cell Transplants to the Cerebral Ventricles Suppress the Pituitary of the Recipient Host. Neuroendocrinology 33:312-316.

Todd, P., W. C. Hymer, L. Plank, G. Marks, E. Kunze and V. Giranda. 1981. Separation of functioning mammalian cells by density gradient electrophoresis. in Electrophoresis '81, eds. Allen, Arnaud, W. De Gruyter, 871-882.

Hymer, W. C. and J. Hatfield. 1983. Separation of cells from the rat anterior pituitary gland. in Methods in Enzymology (M. Conn), eds. Colowick and Kaplan, Academic Press, V. 103, pp. 257-287.

Hymer, W. C., et al. 1984. Hollow fibers: their application to the study of mammalian cell function. in Regulation of Target Cell Responsiveness, ed. K. McKerns, Plenum Publishing Corp. Vol. 1, pp. 407-461.

Hymer, W. C. and J. Hatfield. 1984. Separation of pituitary cells. in Cell Separation: Methods and Selected Applications, eds. T. G. Pretlow and T. Pretlow, Academic Press Vol. 3, pp. 163-194.

Plank, L., W. C. Hymer, M. E. Kunze, G. Marks, J. Lanham and P. Todd. 1983. A Study of Cell Electrophoresis as a Means of Purifying Growth Hormone Secreting Cells. J. Biochem. Biophys. Methods 8:275-289.

\*Barlow, G. H., C. Cleveland, M. A. Farrington, C. Goolsby, R. Grindeland, J. M. Hatfield, W. C. Hymer, M. E. Kunze, J. W. Lanham, M. L. Lewis, D. R. Morrison, B. E. Sarnoff and P. Todd. 1984. Free Flow Electrophoresis of Living Mammalian Cells Under Microgravity Conditions. FEBS Symposium, Moscow, USSR.

\*Morrison, D. R., G. H. Barlow, C. Cleveland, M. A. Farrington, R. Grindeland, J. M. Hatfield, W. C. Hymer, J. W. Lanham, M. L. Lewis, D. S. Hachtway, P. Todd and W. Wilfinger. 1984. Electrophoretic Separation of Kidney and Pituitary Cells on STS-8. COSPAR Invited Paper. G.1.5.2. (in press).

\*Hatfield, M. and W. C. Hymer. 1985. Flow Cytometric Immunofluorescence of Rat Anterior Pituitary Cells. Flow Cytometry. (in press).

G. H. Barlow, C. Cleveland, M. Farrington, R. Grindeland, J. M. Hatfield, W. C. Hymer, J. W. Lanham, M. L. Lewis, D. R. Morrison, P. H. Rhodes, D. Richman, G. V. F. Seaman, R. S. Snyder, G. M. Taylor and W. Wilfinger. Continuous Flow Electrophoretic Separation of Mammalian Pituitary and Kidney Cells. Submitted to Science Feb., 1985.

\*Hymer, W. C., R. Grindeland and J. W. Lanham. 1985. Continuous Flow Electrophoresis (CFE) at Unit and Microgravity: Applications to Growth Hormone (GH) Research and Development. Presented at JPL, Pasadena, CA, Dec. 1984. To be published as part of NRC workshop on microgravity science.

\*Included with report.